

MINIREVIEW

Animal Origins of the Severe Acute Respiratory Syndrome Coronavirus: Insight from ACE2–S-Protein Interactions

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Severe acute respiratory syndrome (SARS) was first described in November of 2002, when inhabitants of Guangdong province, China, presented with an influenza-like illness that began with headache, myalgia, and fever, often followed by acute atypical pneumonia, respiratory failure, and death. The novel disease was transmitted via droplets and fomites and through direct contact of patients with uninfected individuals. The outbreak spread over Asia and to Europe and North America. A total of 8,096 cases were recorded, of which 774 (9.6%) ended in death (11, 54, 72, 108, 111). The etiological agent of SARS was identified as a novel coronavirus (CoV), SARS-CoV (18, 24, 47, 50, 112). This 2002–2003 SARS-CoV epidemic strain was successfully contained by conventional public health measures by July 2003 (71, 110).

SARS-CoV reemerged in Guangdong province in the winter of 2003–2004, when it infected four individuals, all of whom recovered (22, 61, 84). No subsequent human-to-human transmission was observed from these later cases. The infections in 2002–2003 and 2003–2004 are unlikely to be the first instances of SARS-CoV transmission to humans; almost 2% (17 of 938) of serum samples collected in 2001 from one Hong Kong cohort recognized and neutralized SARS-CoV (109). Additional SARS cases resulted from accidental laboratory infections in 2003 and 2004 (62, 70).

Exotic animals from a Guangdong marketplace are likely to have been the immediate origin of the SARS-CoV that infected humans in the winters of both 2002–2003 and 2003–2004. Marketplace Himalayan palm civets (*Paguma larvata*) and racoon dogs (*Nyctereutes procyonoides*) harbored viruses highly similar to SARS-CoV (31). Palm civets are of special interest because the virus could be isolated from most marketplace civets, and SARS-CoV can persist in palm civets for weeks (102). Moreover, the sporadic infections observed in 2003–2004 were associated with restaurants in which palm civet meat was prepared and consumed (61, 84). Additionally, culling of palm civets dramatically reduced the number of infected animals in

the Guangdong marketplace and may be responsible for the absence of the virus in humans after the winter of 2003–2004 (96, 110). Finally, functional studies of the viral receptor, described below, also support a critical role for palm civets in transmitting the virus to humans (60). Evidence of SARS-CoV infection has also been observed in many other marketplace species, including the cat (*Felis catus*), the red fox (*Vulpes vulpes*), and the Chinese ferret badger (*Melogale moschata*) (31, 96).

Although marketplace animals may be the immediate source of the virus found in humans, evidence suggests that they may serve as a conduit for the virus from another reservoir or precursor host. For example, although SARS-CoV antisera and the virus itself were overwhelmingly present in marketplace palm civets in Guangdong, the vast majority of civets on farms and in the wild were found free of infection (46, 74, 92). Further, analysis of the rates of coding changes in the genomes of viruses isolated from palm civets suggests that the genomes are not at equilibrium in the palm civet host (46, 84). Recently, SARS-CoV-like viruses have been isolated from several bat species, predominately horseshoe bats (genus *Rhinolophus*) (53, 59). The genetic diversity of these viruses in bat hosts and the absence of overt disease are consistent with a role for bats as a reservoir for SARS-CoV. However, as described below, substantial genetic changes in the spike (S) protein of bat SARS-CoV are likely necessary for this virus to infect humans.

SARS-CoV isolated from humans can efficiently infect and be transmitted by domestic cats (*Felis domesticus*) and ferrets (*Mustela putorius furo*) (64). BALB/c mice (87, 98), Syrian hamsters (77), and cynomolgus and rhesus macaques (75, 78) are currently being used as animal models for SARS-CoV infection, although transmission has not been observed in these species. Most of these marketplace and laboratory animals, with the notable exception of palm civets, spontaneously clear the virus (64, 102). However, the number of species in which the virus can replicate indicates that SARS-CoV is capable of efficient zoonotic transmission.

Despite this relative ease in interspecies transmission, species variations in host cell factors impose some selection on SARS-CoV-like viruses that are successful in subsequent intraspecies transmission. Advances in our molecular under-

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standing of SARS-CoV cast light on requirements for transmission of this virus from animals, such as bat and palm civet, to humans and for efficient human-to-human transmission. Here we focus on insights gained from study of the SARS-CoV spike (S) protein, which mediates viral entry, and its interaction with angiotensin-converting enzyme 2 (ACE2), the cellular receptor for SARS-CoV (48, 58).

CORONAVIRUSES

Coronavirus virions contain one copy of a 27- to 32-kb-long, capped, and polyadenylated single-stranded RNA of positive polarity, which is helically encapsidated by nucleocapsid proteins (6, 52). This unusually large genome—29 kb in the case of SARS-CoV—may reflect reduced dependency on cellular proteins, a property useful in efficient cross-species transmission. The filamentous ribonucleocapsids are surrounded by matrix proteins, which form the virus cores. These cores are wrapped in envelopes formed during coronavirus budding from a host cell. The membranes contain distinct club- or petal-shaped protrusions identified as S proteins. It is these proteins that give the virions a crown-like appearance (Latin, *coronae*) in electron microscopic images. S proteins are the major antigenic determinants of coronaviruses, and as described below, they mediate receptor association and fusion of the viral and cellular membranes (15, 25, 42, 52).

Three distinct genetic and serological groups of coronaviruses have been defined, but this grouping is undergoing revision (6, 27). Coronaviruses from groups 1 and 2 are known to cause disease in humans (67). Human coronavirus 229E (HCoV-229E), a group 1 virus, and human coronavirus OC43 (HCoV-OC43), a group 2 virus, cause mild upper respiratory tract infections that result in self-resolving common colds in otherwise healthy individuals or severe pneumonia in immunocompromised people (6, 67). Human coronavirus NL63 (HCoV-NL63; also referred to as HCoV-NH and HCoV-NL) has recently been identified as a group 1 virus causing conjunctivitis, croup, and, sometimes, serious respiratory infections in children (21, 23, 94). HCoV-NL63 is also notable for its use of the SARS-CoV cellular receptor ACE2 to infect cells (40). Another group 2 coronavirus (HCoV-HKU1) was recently isolated from a 71-year-old man with pneumonia (101). SARS-CoV and SARS-CoV-like viruses found in animals also cluster with group 2 viruses, although they are outliers of group 2 and have also been described as group 4 or, more recently, group 2b viruses (26, 28, 83).

Like that of other RNA viruses, coronavirus diversity is generated by mutations due to polymerase infidelity. In addition, a key feature of coronavirus evolution is the propensity of the viral genomic RNA to recombine (41). Recombination permits the virus to acquire genes and gene regions from other transcripts, including those of other coronaviruses. Targeted recombination has been effectively used in the laboratory to manipulate and study coronavirus genomes (7, 32, 51, 55, 79). Natural recombination permits the rapid transformation of viral proteins such as the S protein. For example, acquisition of a small region of the S protein by a SARS-CoV precursor perhaps originally more similar to the SARS-CoV found in bats may have allowed it to utilize ACE2. In general, recom-

bination can alter the tissue tropism of a virus and provide new avenues for further evolution and interspecies transmission.

CORONAVIRUS S PROTEINS AND THEIR RECEPTORS

The host spectrum of a specific coronavirus is largely determined by its S protein (51, 73, 79). In many cases, subtle alterations of the S protein are sufficient to alter tissue and species tropism and the virulence of a coronavirus (7, 32, 51, 80). Coronavirus S proteins are type I transmembrane and class I fusion proteins that consist of distinct N-terminal (S1) and C-terminal (S2) domains, which mediate receptor binding and virus-cell fusion, respectively (4, 15, 25). Following association with the cell surface receptor, the S protein undergoes a conformational change that exposes a fusion peptide embedded in the S2 domain and induces reorganization of S2's large heptad repeats into coiled coils. This conformational change brings the virion membrane into close apposition to the cellular membrane for subsequent fusion (12, 15, 52).

Some coronavirus S proteins, for example, that of murine hepatitis virus, are cleaved between their S1 and S2 domains by a furin-like protease in the producer cell (44, 85, 86). Others, for example, those of HCoV-229E and SARS-CoV, do not retain furin recognition sites and are uncleaved on the virion (1, 68, 103). SARS-CoV is nonetheless dependent, following receptor association, on protease activity in the target cell (66, 82). This proteolysis can be mediated by cathepsin L in an endosomal or lysosomal compartment or by exogenous proteases such as trypsin, thermolysin, and elastase. The role of proteolysis in the target cell remains to be determined, but it is likely that its function may be distinct from that of furin cleavage in the producer cell. For example, filovirus GP_{1,2} proteins, analogous to coronavirus S proteins, retain dependency on cathepsin B and L despite their cleavage into GP₁ and GP₂ in the producer cell (9). Not all coronaviruses are dependent on cathepsins or other lysosomal cysteine proteases; infection by HCoV-NL63 is not dependent on these enzymes, despite its utilization of the same receptor as SARS-CoV (42a). Variation in cathepsin activity may, like receptor expression, govern the efficiency of infection in different tissues.

Several coronavirus cell surface receptors have been identified. Aminopeptidase N (CD13) was shown to be the receptor for canine coronavirus, feline infectious peritonitis virus, HCoV-229E, porcine epidemic diarrhea virus, and transmissible gastroenteritis virus, all of which are group 1 coronaviruses (14, 107). Members of the pleiotropic family of carcinoembryonic antigen-cell adhesion molecules (CEACAMs) were identified as receptors for the group 2 pathogen murine hepatitis virus (19, 20, 99), whereas bovine group 2 coronaviruses bind to 9-O-acetylated sialic acids (81). In 2003, ACE2 was identified as a functional cellular receptor for SARS-CoV (58). The role of ACE2 in HCoV-NL63 infection was demonstrated following isolation and characterization of this recently described group 1 coronavirus (40).

ACE2, THE SARS-COV RECEPTOR

ACE2 was identified as a functional receptor for SARS-CoV, using a direct biochemical approach (58). The S1 region of the SARS-CoV S protein was used to precipitate ACE2

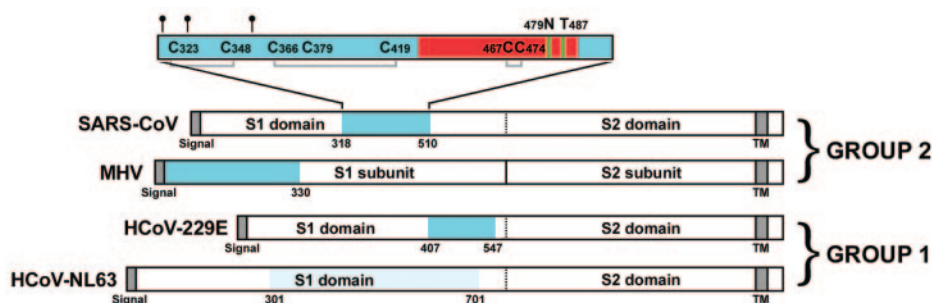


FIG. 1. Receptor-binding domains of coronaviruses. A representation of the primary structures of SARS-CoV, mouse hepatitis virus (MHV), and two group 1 human coronaviruses (HCoV-NL63 and HCoV-229E) is shown. The receptor-binding domains of each coronavirus are shown in cyan. The RBD of HCoV-NL63 is preliminary. The SARS-CoV RBD is shown in greater detail, with glycosylation sites and the disulfide structure indicated. Red denotes the receptor-binding motif within the RBD. The RBM comprises a long loop in direct contact with ACE2.

from Vero E6 cells, an African green monkey kidney cell line previously shown to support efficient viral replication. Robust syncytia formed between HEK 293T cells expressing the S protein and those overexpressing ACE2. Transfection of cell lines with ACE2 rendered them permissive to infection with SARS-CoV and with retroviruses pseudotyped with S protein (58, 68). Anti-ACE2 antisera, but not identically prepared anti-ACE1 sera, blocked replication of SARS-CoV, as did a soluble form of ACE2.

Many lines of evidence further implicate ACE2 as the principal receptor utilized *in vivo* by SARS-CoV. ACE2 is expressed in the lung and in the gastrointestinal tract, the major sites of replication of the virus (8, 16, 33, 34). The efficiency of infection in humans, mice, rats, and palm civets correlates with the ability of the ACE2 of each species to support viral replication (57, 60, 87, 98, 102). ACE2 binds S protein specifically, with approximately 2 nM affinity (88). Although many cell lines do not express ACE2, all cell lines shown to support efficient SARS-CoV infection express this receptor (39, 69). The ACE2-binding region of the S protein raises a protective neutralizing antibody response in mice, and anti-S-protein antibodies that block ACE2 association protect mice and hamsters against infection (30, 36, 38, 89). Finally, little or no viral replication is observed in ACE2^{-/-} mice (48). Additional factors may also contribute to the efficiency of infection. DC-SIGN-related protein (DC-SIGNR; L-SIGN, CD209L), DC-SIGN (CD209), and L-SECTin have been shown to enhance infection of ACE2-expressing cells (29, 45, 65, 105). These proteins do not appear to mediate efficient infection in the absence of ACE2 (45, 65). As previously described, cathepsin L or other alternative proteases are also necessary for efficient infection following ACE2 association (66, 82).

ACE2 is a type I transmembrane protein with a single metalloprotease-active site with a HEXXH zinc-binding motif (17, 90). The physiological function of ACE2 remains unclear. The enzyme has been shown to cleave a variety of regulatory peptides *in vitro*, among them angiotensin I and II, des-Arg-bradykinin, kinetensin, and neurotensin (17, 95). Some cleavage products have been shown to be potent vasodilators with antidiuretic effects. This finding suggests that ACE2 counterbalances the actions of ACE1, which mediates vasoconstriction (104). Furthermore, targeted disruption of ACE2 in mice resulted in severe cardiac contractility defects (13). The enzy-

matic activity of ACE2 does not contribute to its ability to mediate fusion and viral entry, and small molecule inhibitors that block catalysis do not inhibit SARS-CoV infection (60). However, ACE2 proteolysis has been implicated in SARS pathogenesis and in acute respiratory distress syndrome (ARDS). These studies also demonstrated that SARS-CoV S protein can down-regulate pulmonary ACE2 and that soluble ACE2 can protect mice from lung injury in a model of ARDS.

S-PROTEIN RECEPTOR-BINDING DOMAINS

Discrete, independently folded, receptor-binding domains (RBDs) of the S proteins of several coronaviruses have been described (2, 3, 5, 49, 100, 103). The first 330 amino acids of the 769-residue S1 subunit of the murine hepatitis virus S protein is sufficient to bind its receptor, CEACAM1 (49). A very different region of the S1 domain of HCoV-229E, between residues 407 and 547, is sufficient to associate with CD13 (3, 5). A 192-amino-acid fragment of the SARS-CoV S1 domain, residues 319 to 510, binds human ACE2 with greater efficiency than does the full-length S1 domain (2, 100, 103). As shown in Fig. 1, the RBDs of these coronaviruses are found in distinct regions of the primary structure of the S protein. This pattern may suggest that coronavirus S proteins are adapted for easy acquisition of novel binding domains or for rapid shifts in receptor usage.

The crystal structure of the SARS-CoV RBD is consistent with this speculative possibility (56). The RBD contains two subdomains—a core and an extended loop (Fig. 2). The core is a five-stranded, antiparallel β -sheet, with three short connecting α -helices. The loop, residues 424 to 494, termed the receptor-binding motif (RBM), is the only domain that contacts ACE2 directly. Although the RBD core domain is homologous with similar regions of other group 2 coronaviruses, the RBM is unique to SARS-CoV (Fig. 2). The RBM may have been acquired from another coronavirus, perhaps a group 1 virus relative of HCoV-NL63. As indicated, HCoV-NL63 also enters cells through ACE2 (40), and its extended RBD region includes a stretch of residues with weak homology to the SARS-CoV RBM (unpublished observations).

Moreover, the recently described SARS-CoV-like viruses isolated from bats lack this stretch of residues, including most residues directly contacting ACE2 (Fig. 3) (53, 56, 59). The absence of these RBM residues is consistent with the inability

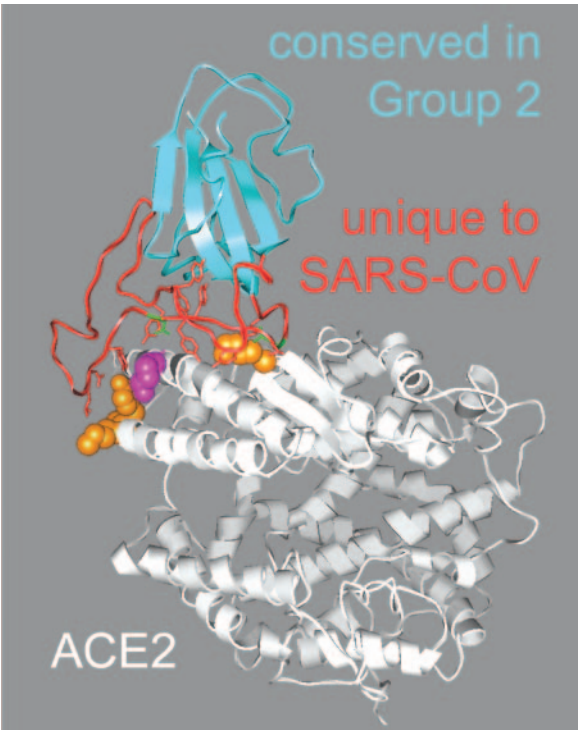


FIG. 2. Cocrystal of the SARS-CoV RBD bound to human ACE2. Cyan indicates a region of the RBD shared among group 2 coronaviruses, whereas red indicates the RBM, which is not homologous to that of other group 2 viruses. ACE2 is shown in white, with its cleft bearing the enzyme-active site facing forward and the membrane-associated C terminus at the bottom of the figure.

of these viruses to grow on tissue culture cells permissive for SARS-CoV (53, 59). If indeed bats are reservoir animals for a SARS-CoV predecessor, acquisition of this ACE2-binding region is likely to have been a critical event in the evolution of the virus. According to this scenario, the virus found in bats utilizes another receptor. A recombination event that occurred in bats, palm civets, or another host, perhaps with a group 1 virus similar to HCoV-NL63, may have given rise to SARS-CoV.

Humoral responses are sufficient to protect animals from SARS-CoV infection. A number of independent studies have found the RBD to be the major immunodominant and a potent neutralizing epitope on the S protein (10, 36, 38, 91, 93, 97, 113). Inoculation of the RBD induces potent neutralizing-antibody responses in rabbits and mice (37) and appears to

completely protect mice from SARS-CoV challenge (Dale Barnard, personal communication). Neutralizing antibodies against the SARS-CoV RBD are relatively easy to generate, consistent with exposure of this domain (88, 113). Monoclonal antibodies targeted to the SARS-CoV RBD are effective in protecting mice at doses usable in humans (30, 89, 91). Collectively, the data suggest that the SARS-CoV RBD readily elicits antibodies that block replication. The exposure of this domain may reflect its recent acquisition or a strategy in which rapid transmission is favored over immune escape.

THE S-PROTEIN-BINDING REGION OF ACE2

The ability of the ACE2 proteins of mice, rats, and palm civets to support SARS-CoV infection has been compared with that of human ACE2 (57, 60). SARS-CoV infection was less efficient in cells expressing murine ACE2 than in cells expressing human ACE2. Infection was nearly absent in those expressing rat ACE2. Consistent with a role for palm civets in transmitting virus, palm civet ACE2 supported SARS-CoV infection as efficiently as human ACE2. These results correlated with the affinity of each of these receptors for the S protein and its RBD (57, 60). Chimeras of human and rat ACE2 receptors were used to identify the S-protein-binding site on ACE2 (60). Alteration of four rat ACE2 residues (82 to 84 and 353) to their human equivalents converted rat ACE2 to an efficient SARS-CoV receptor. Residues 82 to 84 comprise a glycosylation site on the rat receptor that is not present on the mouse, palm civet, or human receptor. Residue 353 is a histidine in mouse and rat receptors and a lysine in palm civet and human ACE2. Strikingly, alteration of histidine 353 of mouse ACE2 to the human lysine results in a receptor that supports infection as efficiently as human ACE2 (W. Li, unpublished observation). Alterations of additional residues along the first helix of human ACE2 (lysine 31 and tyrosine 41) to alanine interfered with S-protein-mediated infection and RBD association. Collectively these data localize the S-protein-binding region to the membrane-distal lobe of the cleft that contains the catalytic site of ACE2 (56, 60).

S-PROTEIN VARIATION IN HUMAN AND ANIMAL ISOLATES

Three S proteins of distinct origins have been compared for their ability to use human and palm civet ACE2 (60, 76, 106). The first, TOR2, was isolated during the 2002-2003 epidemic (63). The second, designated GD03, was isolated from the

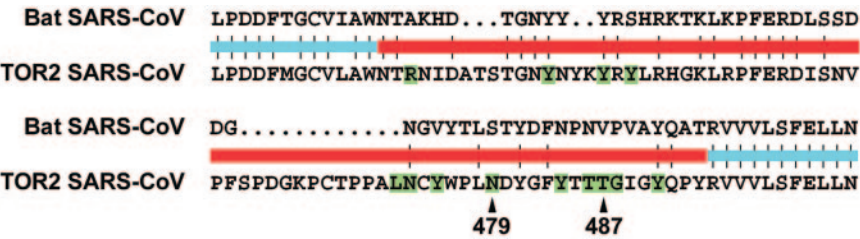


FIG. 3. Bat SARS-CoV lacks an ACE2 RBM region. Alignment of a portion of the TOR2 SARS-CoV RBD with the equivalent region of bat SARS-CoV is shown. The RBM region is indicated in red. Residues that directly contact human ACE2 are shown in green, and residues 479 and 487 are indicated with arrows.

Predominant S-protein amino acids:			
S-protein residue #	SARS-CoV from 2003-2004 (e.g. GD03)	SARS-CoV from palm civets (e.g. SZ3)	SARS-CoV from 2002-2003 (e.g. TOR2)
479	N	K	N
487	S	S	T

Most efficient ACE2 association:			
S-protein residue #	Reservoir ACE2	Palm civet ACE2	Human ACE2
479	K?	K=N	N
487	S?	T	T

FIG. 4. Summary of genetic and biochemical studies of SARS-CoV S-protein residues 479 and 487. (Top) The most frequently observed residues at positions 479 and 487 in sequences of viral genomes obtained during the 2002-2003 human SARS-CoV epidemic and the sporadic infections of 2003-2004 and from palm civets in a Guangdong marketplace. Note that a single isolated palm civet genome (from >20 sequences) encodes a threonine at 487, whereas all sequences from the 2002-2003 epidemic (>100 sequences) encode this threonine. (Bottom) The S-protein residues that confer the most-efficient binding to the ACE2 proteins of the indicated species. The entry for reservoir species is speculative, based on the observation that the ACE2 of at least one animal (mouse) prefers lysine at residue 479 and the additional observation that all but one sequence from the Guangdong marketplace animals encode a serine at residue 487.

sporadic infections in 2003-2004 (35). The third, SZ3, was obtained from palm civets (31). Both SZ3 and, less expectedly, GD03 bound and utilized palm civet ACE2 much more efficiently than human ACE2 (60). In contrast, TOR2 utilized both receptors efficiently. The efficiency with which virus from both human outbreaks utilized palm civet receptor is consistent with the recent transfer of SARS-CoV from palm civets to humans. The lower efficiency with which GD03 utilized human ACE2 compared with TOR2 may in part account for the mildness of symptoms and the absence of subsequent transmission observed during the 2003-2004 infections (61, 84).

The differences in these three S proteins were also reflected in the ability of their RBDs to bind human and palm civet ACE2 (Fig 4). Two amino acids, residues 479 and 487, largely determined the much greater efficiency with which the TOR2 RBD bound human ACE2 (60, 76). Residue 479 is an asparagine or serine in all S proteins isolated from humans during either the 2002-2003 epidemic or the 2003-2004 infections. However, most sequences isolated from palm civets or raccoon dogs encode a lysine at this position. This lysine is incompatible with human ACE2, but palm civet ACE2 can efficiently bind S proteins expressing either lysine or asparagine without an apparent preference for either (60). Palm civets may therefore be an important intermediate in the transfer of SARS-CoV to humans, permitting the emergence of viruses that express a small, uncharged amino acid at S-protein residue 479.

Residue 487 is also of interest. Residue 487 is a threonine in all of the more than 100 S-protein sequences obtained during the 2002-2003 outbreak (35). It is a serine in S proteins from viruses isolated during the mild 2003-2004 infections and in all but one of the 20 or so S-protein sequences obtained from palm civets and raccoon dogs. The relatively modest change of threonine to serine in the TOR2 RBD resulted in an approximately 20-fold decrease in binding to human ACE2 (60). A corresponding increase was observed when a threonine was introduced into the SZ3 RBD. A threonine at position 487 also substantially increased association with palm civet ACE2. Notably, the single palm civet-derived S-protein sequence that

encoded a threonine at position 487 also encoded an asparagine at position 479 (Zhihong Hu, personal communication). The emergence of this rare combination of S-protein residues in the palm civet-derived virus may have been necessary to generate a SARS-CoV that could efficiently transmit between humans. The infrequency of threonine 487 in animal-derived viruses may suggest that the receptor of the ultimate reservoir of SARS-CoV better utilizes a serine at this position.

The recently published cocrystal of ACE2 with the SARS-CoV RBD clarifies these observations (56). TOR2 S protein asparagine 479, most commonly a lysine in palm civet virus, interacts with a network of residues that include lysine 31 of human ACE2 (Fig. 5). Palm civet and murine ACE2s express small, uncharged residues at this position, presumably better

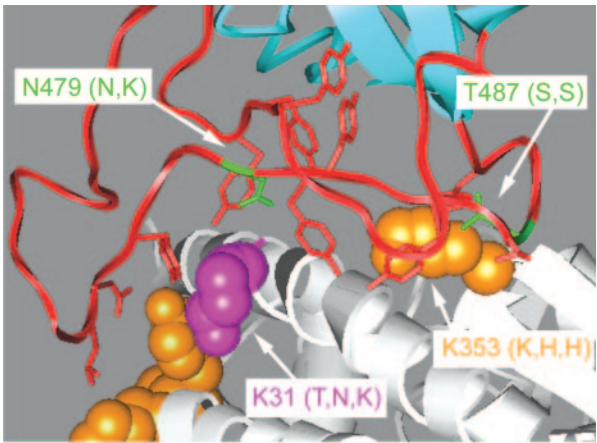


FIG. 5. The contact region between the SARS-CoV RBD and ACE2 is shown. Residues that convert rat ACE2 to an efficient receptor are shown in orange. ACE2 lysine 31, which prevents association with SZ3 S protein, is shown in magenta. Lysine 31 and lysine 353 are indicated by arrows, with the amino acids of palm civet, mouse, and rat ACE2 at these positions shown in parentheses. TOR2 S-protein residues asparagine 479 and threonine 487 are also indicated, with the GD03 and SZ3 amino acids at these positions shown in parentheses.

accommodating an S-protein lysine. S-protein residue 487, a threonine in all epidemic SARS-CoV isolates, directly contacts critical ACE2 lysine 353 (Fig. 4). Interaction of the threonine methyl group with lysine 353 provides a clear explanation for the decrease in affinity for human and palm civet ACE2 when this threonine is altered to serine.

CONCLUSIONS

The intense scientific effort expended in describing SARS and SARS-CoV has provided a unique case study in viral evolution and zoonotic transmission. The SARS-CoV example underscores the need in some instances for a bridge species that is in direct contact with humans and that may guide virus evolution so as to permit emergence of a variant that can transmit efficiently among humans. It highlights viral strategies that permit rapid adaptation to new species and shows that mildly pathogenic viruses may not remain so with changes in human and animal populations which increase viral diversity or the frequency of cross-species contacts. Experience with SARS-CoV has demonstrated the importance of field work that identifies and characterizes viruses and host factors in wild and domesticated animals. Further work in these directions may help anticipate and avoid the next SARS.

Important questions remain. What receptor does bat SARS-CoV utilize? If bats are indeed a reservoir of SARS-CoV-like viruses, when and in which species did these viruses acquire an S protein capable of using palm civet and human ACE2? Did SARS-CoV gain the use of ACE2 through recombination, and if so, with what virus? Are changes in the S protein that enhanced human-to-human transmission a probable consequence of incubation in palm civets and other animals or a unique event unlikely to recur? What changes in other viral proteins were necessary for SARS-CoV to transmit efficiently among humans? Study of SARS-CoV suggests we should direct our attention to adaptation strategies themselves, such as reassortment (for influenza viruses) and recombination (for coronaviruses), rather than to the end products of these adaptations. It also suggests that further attention should be given to the possible emergence of dangerous variants of common pathogens, such as HCoV-NL63 and its animal equivalents. In short, there are more lessons to learn from SARS.

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